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Evaluation of Biochemical and Physiological Parameters of the Leaves of Tree Species Exposed to Vehicular Emissions

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Authors' contributions

This work was carried out in collaboration between all authors. Author DGO designed the study, carried out the experiment, managed the literature searches and wrote the first draft of the manuscript. All other authors managed the analyses and interpreted the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the sensitivity and tolerance levels of the tree species to air pollution using biochemical and physiological parameters.
Study Design: Field and Laboratory Studies
Place and Duration of the Study: Three major roads in Ile-Ife, Institute of Ecology and Environmental Studies Laboratory and Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Osun State, Southwest, Nigeria.
Methodology: The air pollution tolerance indices (APTI) of twelve dominant plant species found along three major roads in Ile-Ife, Osun State, Southwest Nigeria were studied, with a view to determining their sensitivity to air pollution. Biochemical and physiological parameters such as, relative water content, total chlorophyll, leaf extract pH and ascorbic acid concentration of the

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leaves of the selected trees were analyzed to determine their APTI.
Results: The lowest pH value was obtained in *Spondias mombin* (2.75) while *Albizia zygia* had the highest pH value (5.47). Ascorbic acid concentration of the leaves ranged within 3.14 mg/g and 7.88 mg/g, with the lowest and highest value recorded in *Psidium guajava* and *Polyalthia longifolia* respectively. The total chlorophyll of the leaves of the studied plants was estimated to be within the range of 0.41 mg/g and 0.80 mg/g, with the highest value in *Anacardium occidentale* and the lowest in *Tectona grandis* while relative water content of leaves ranged between 74.4% (*Senna siamea*; lowest) and 90.5% (*Terminalia catappa;* highest). The APTI of the tree species ranged between 9.2 and 12.7, the highest value was obtained in *Polyalthia longifolia* and the lowest value in *Psidum guajava*.
Conclusion: *P. longifolia, M. indica, G. arborea, T. grandis and T. catappa* were the most tolerant to air pollution of all the tree species.

Keywords: Air pollution; biochemical parameters; environment; green belt; tree species.

1. INTRODUCTION

Biochemical and physiological parameters of plants parts are important in determining their sensitivity and tolerance to pollution and other adverse environmental factors. Trees within the urban centers perform various environmental functions apart from their aesthetic values. Vehicular pollution is one of the greatest menaces facing many people in recent times, especially in a developing nation like Nigeria. Air pollution is defined as a fluctuation in any atmospheric constituent from the value that would have existed without human activity [1]. The increased level of pollution is very much connected to the number of vehicles that is steadily rising, leads to concurrent increase in pollution. It is perceived in Nigeria that the number of vehicles has increased and all vehicles burn petrol or diesel to move around which release gases that cause several injuries to both man and flora. Vehicular emission represents a major source of air pollutants as CO, SO_2, NO_2, and particulate matters that affect plant growth adversely [2]. These pollutants have been observed to cause damage to leaf cuticles affecting stomata conductance, photosynthetic systems, leaf longevity and patterns of carbon allocation within plants [3]. The urban air quality is continuously affected by emissions from both stationary and mobile combustion sources and this urgently requires serious attention. Due to the environmental benefit associated with planting of tress, it was concluded that urban environments rely on vegetation to provide ecosystem functions such as air filtering, temperature amelioration, water storage filtration and drainage [4]. The vegetation of urban centers has societal values which define nature for millions of people living in cities and sustaining their well-being [5].

The response of plants to air pollution at physiological and biochemical levels can be understood by analyzing the factors that determine resistance and susceptibility [6]. Chlorophyll is the photosynthetic pigment in plants. Its content in plants varies from species to species, depending on the leaf age, pollution level as well as with other biotic and abiotic conditions [7]. Chlorophyll is not a single molecule but a family of related molecules, designated as chlorophyll ‘a’, ‘b’, ‘c’, and ‘d’. Ascorbic acid, also called vitamin C is a strong reductant and it activates many physiological and defense mechanism in the plants [8]. It reducing power is directly proportional to its concentration [9]. Ascorbic acid is also pH dependent, being more at higher pH levels because high pH increases the efficiency of conversion of hexose sugar to ascorbic acid and this is related to pollution tolerance [10,11]. Apart from this, relative water content (RWC) of leaves can also be examined to reveal tolerance level of plants to stressful conditions. Therefore, analysis of biochemical parameters of tree species growing in polluted areas is of utmost necessity to identify tree species that could be promoted in afforestation in urban cities to absorb pollutants and curtail air pollution. Thus, this study therefore aimed at evaluating biochemical and physiological parameters of tree species along major roads in the study area and identifying air pollution tolerant species that can be used to line the major city roads in the tropics.

2. MATERIALS AND METHODS

2.1 Sampling Procedure

Twelve tree species were selected from three major roads within Ile-Ife city; they are Ife-Ibadan, Ife-Ilesa and Ife-Ondo roads. They include *Gmelina arborea, Mangifera indica, Tectona grandis, Polyalthia logifolia, Anacardium occidentale, Newbouldia laevis, Terminalia catappa, Spondias mombin, Senna siamea, Gliricidium sepium, Albizia zygia, and Psidium guajava.* The criterion for the selection of these tree species was mainly dominance in the study areas, the tree species were within a distance of 0 to 15 m of both sides of the road with height and girth greater than or equal to 1.3 m and 10 cm respectively. Also, the choice of these routes was based on their high traffic density. Fully matured leaves from triplicates of the tree species were collected and taken to the laboratory in a heatproof container for analysis.

2.2 Biochemical Analyses of Leaves

2.2.1 Estimation of relative water content

In order to estimate the leaf relative water content, fresh and matured leaves from triplicates of the tree species were collected and quickly transferred to the laboratory and weighed on a weighing balance (Model Scout Pro SPU 2000) to obtain the fresh weight (FW). Thereafter, the leaves were immersed in water for 24 hours, blotted dry and later weighed to obtain the turgid weight (TW). Finally, the leaves were dried in an oven for 2 days at 70℃ and reweighed to obtain the dry weight (DW) [12]. The relative water content was determined as follows:

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100$$

2.2.2 Estimation of total leaf chlorophyll

Three grammes of fresh leaves were blended and diluted with 10 ml of 80% acetone [11]. After 15 minutes, the liquid portion was decanted into another test-tube and centrifuged at 2,500 rpm for 3 minutes using Harrier 15/80 Table top centrifuge. The optical density of the collected supernatant was read at 645 nm and 663 nm using a Bio Quest UV spectrophotometer Cecil 2000 series.

Optical density (C_T) of the total chlorophyll is the sum of the chlorophyll a (D_{645}) and chlorophyll b (D_{663}).

$$CT = 20.2\,(D645) + 8.02(D663)$$

Total leaf chlorophyll was calculated thus:

$$Total\ chlorophyll\ (mg/g\ DW) = 0.1\,CT \times (Leaf\ DW/Leaf\ FW)$$

0.1 is a constant.

2.2.3 Estimation of leaf ascorbic acid concentration

Ascorbic acid content of the leaf (expressed in mg/g) was measured spectrophotometrically. To the fresh foliage (1 g) in a test-tube was added, 4 ml extracting solution [oxalic acid EDTA (Ethylenediaminetetraacetic acid)], then 1 ml of orthophosphoric acid, 1 ml of 5% tetraoxosulphate (vi) acid, 2 ml of ammonium molybdate and 3 ml of distilled water [13]. The reaction mixture was allowed to stand for 15 minutes and absorbance was taken at 760 nm with a Bio Quest UV spectrophotometer Cecil 2000 series. The concentration of ascorbic acid in the sample was then extrapolated from ascorbic acid standard curve.

2.2.4 Determination of leaf extract pH

The leaf extract pH was determined following the method of [14]. Fresh leaves (5 g) were homogenized in 50 ml deionized water and filtered with a Whatman 42 filter paper. The pH of the leaf extract was measured with pH meter (Model PHS – 3B) at room temperature.

2.3 Air Pollution Tolerance Index Calculation

The air pollution tolerance index (APTI) of different plant species were calculated by incorporating pH, ascorbic acid, total chlorophyll and relative water content into formula below [10].

$$\mathrm{APTI} = \frac{\mathrm{A}\,(\mathrm{T} + \mathrm{P}) + \mathrm{R}}{10}$$

Where: A is ascorbic acid (mg/g DW); T is total chlorophyll (mg/g DW); P is pH of the leaf extract; R is relative water content of leaf (%) and 10 a constant value.

Using values of APTI, plants are classified into three groups [10] as shown in Table 1.

Table 1. APTI values and responses of plant species

APTI	Value	Responses
A	<11-11.9	Sensitive
B	12 – 16	Intermediate
C	17 and above	Tolerant

2.4 Statistical Analysis

Statistical analyses of data collected during the experiment were carried out using software SPSS 20.0. The data were analyzed with Graphical tools and Correlation and results were considered significant at 95% Confidence Interval.

3. RESULTS AND DISCUSSION

The results of the four biochemical and physiological parameters studied and the APTI values among the tree species are presented in Figs. 1 to 5.

3.1 Leaf Extract pH

The pH values obtained ranged between 2.75 and 5.47. The lowest value was obtained in *Spondias mombin* while the highest was obtained in *Albizia zygia*. All the studied plant leaves sampled exhibited a shift in pH towards acidic range as presented in Fig. 1. The acidic nature may be due to the presence of SO_2, NOx and other acidic pollutants emitted from vehicles into the ambient air [15,16]. It was reported that high pH improves plant tolerance to air pollution [17]. Acidic gaseous pollutants from vehicles diffuse and form acid radicals in the leaf matrix by reacting with cellular water [18]. Diffusion of SO_2 through stomata, dissolves in water to form sulphites, bisulphate and their ionic species which lead to proton generation and eventually changes the cellular pH [15].

3.2 Ascorbic Acid Concentration

The result of ascorbic acid concentrations of the leaves is shown in Fig. 2, values were within 3.14 mg/g and 7.88 mg/g. The lowest value was recorded in *Psidium guajava*, while the highest was obtained in *P. longifolia*. The relatively low concentrations of ascorbic acid found in the leaves of *P. guajava* suggest its sensitive nature to air pollution particularly resulting from automobile exhaust. This agrees with the findings of [19] which stated that lower ascorbic acid concentration in the leaves of plant species supports their sensitive nature. Ascorbic acid is a very important reducing agent, it plays a vital role in cell wall synthesis, cell division and activates the defense mechanism of the plant [19]. The level of this acid can therefore be expected to decline on pollutant exposure. Thus, plants maintaining high ascorbic acid level even under polluted conditions are considered to be tolerant to air pollutants since its reducing power is directly proportional to its concentration. Soil contamination and air pollution have also been found to result in decrease in leaf ascorbic acid concentration in exposed *Tibouchina pulchra* saplings [20], and this together with mineral deficiencies, are among the factors responsible for the formation of reactive oxygen species (ROS) [21]. Given that ascorbic acid decreases reactive oxygen species concentration in leaves, increased levels of ascorbic acid in leaves will help increase air pollution tolerance in plants [22].

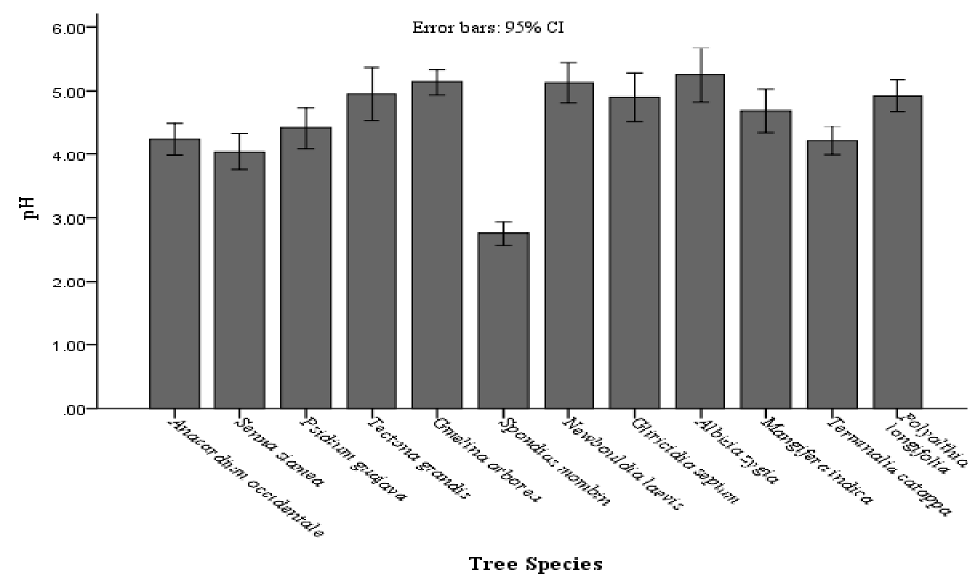

Fig. 1. pH of the leaves of selected tree species

3.3 Total Chlorophyll Content

The results of total chlorophyll content of the leaves are shown in Fig. 3, values were within 0.41 mg/g and 0.80 mg/g. The highest value was obtained in *Anacardium occidentale* while the lowest was obtained in *Tectona grandis*. High chlorophyll content in plants is reported to favour tolerance to pollutants [23], whereas a considerable loss in total chlorophyll in the leaves of plants may occur when exposed to air pollution stress. This supports the argument that the chloroplast is the primary site of attack by air pollutants such as suspended particulate matter, SO_2 and NO_x released from automobile exhaust [24]. Furthermore, the decrease in chlorophyll content may also be due to the alkaline condition created by the dissolution of chemicals present in dust particles in the cell sap, which is responsible for chlorophyll degradation [25].

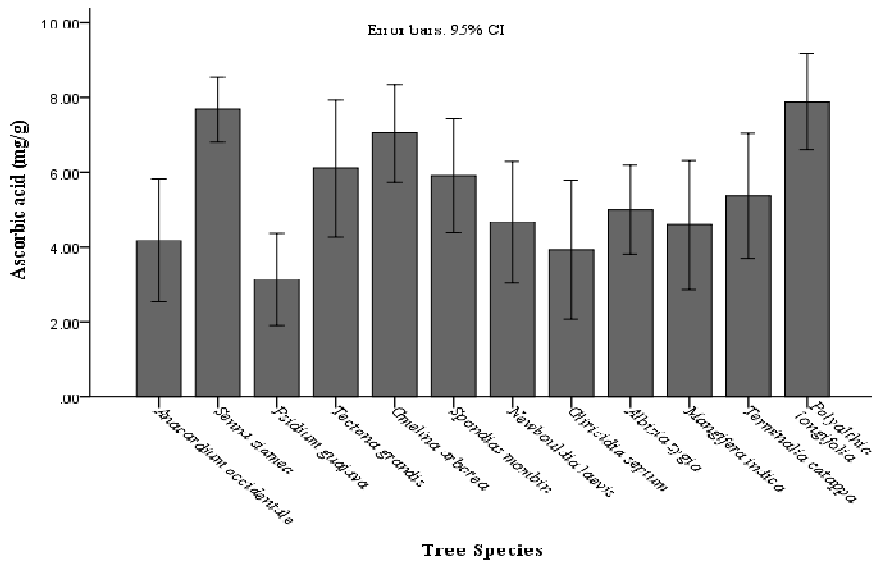

Fig. 2. Ascorbic acid of the leaves of selected tree species

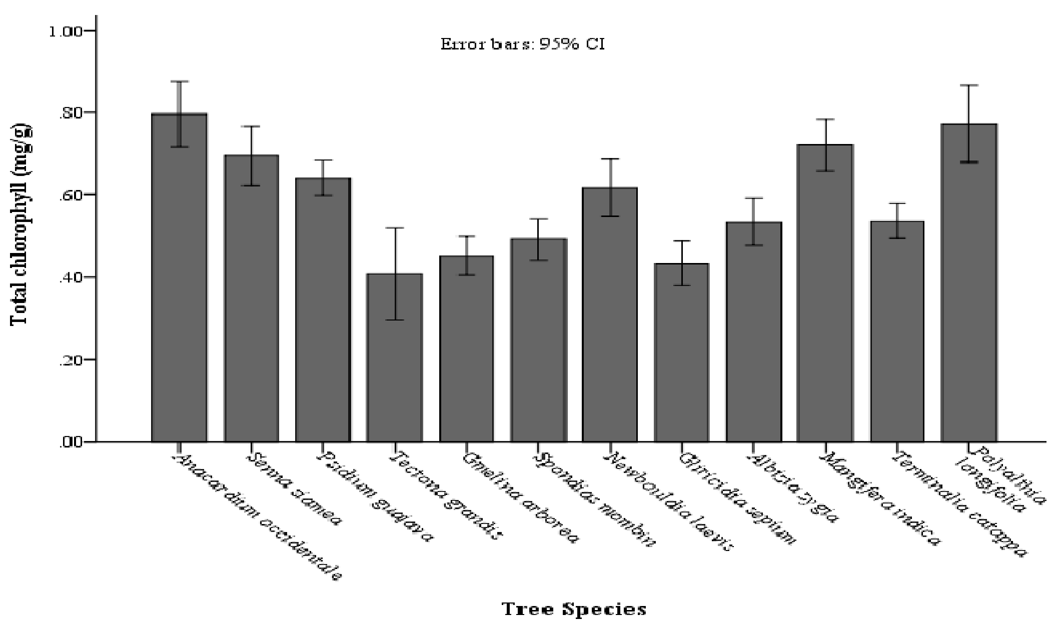

Fig. 3. Total chlorophyll of the leaves of selected tree species

3.4 Relative Water Content

The results of the relative water content of the leaves are shown in Fig. 4, values ranged between 74.4% and 90.5%. The highest value was obtained in *Terminalia catappa* while the lowest value was obtained in *Senna siamea*. The relative water content (RWC) of a leaf is associated with protoplasmic permeability in the cells and high water content within a plant body prevents desiccation under adverse weather conditions. Therefore, maintenance of relative water content in the tissues of a plant is one of the factors determining its relative tolerance to air pollution [26]. An average RWC ranging from 58.1 to 99.8% in the tree species studied in polluted sites of the industrial town of Bhadravathi, India [16]. The results from this study also agree with the foregoing that plants growing in polluted sites retain more water than those in unpolluted sites. A plausible explanation to this might be that the plants at the polluted areas absorbed more water as an adaptive feature which helps in maintaining its physiological balance against pollution stress [27]. It might also be indications that plants with high relative water content absorb pollutants that are hydrophilic, that is, have affinity for water. This feature enabled the plants to retain more water content in polluted conditions which makes them tolerant to pollution stress. Low relative water content of leaf means lower rate of availability of water in soil along with high rate of transpiration.

3.5 Correlation Analysis

The correlation matrix shows the relationship that existed among the four biochemical parameters studied and the air pollution tolerance index. A significant positive correlation existed between pH and APTI (0.296) at $p<0.01$. A strong positive correlation also existed between ascorbic acid and APTI (0.803) at $p<0.01$ and between relative water content and APTI (0.521) at $p<0.01$. A positive correlation existed between ascorbic acid and total chlorophyll (0.147) at $p<0.05$ (Table 2). Consequently, it can be stated that each parameter plays a significant role in the computation of the air pollution tolerance index. In this study, a positive correlation exists between air pollution tolerance index and pH, ascorbic acid and relative water content ($p<0.01$). Nevertheless, a strong positive correlation exists between APTI and ascorbic acid and relative water content. This also agrees with the findings of a research carried out in a similar urban area that a high positive correlation ($p<0.01$) exists between APTI and ascorbic acid and relative water content [18]. This present study indicates that ascorbic acid and relative water content of leaves are among the most significant and determining parameters on which tolerance depends.

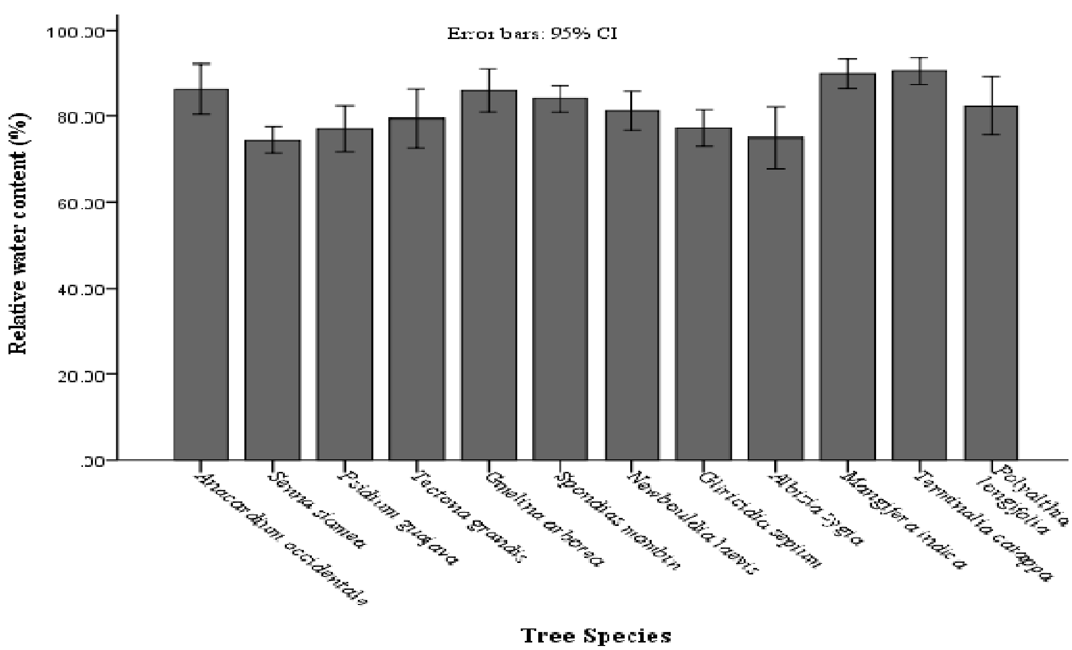

Fig. 4. Relative water content of the leaves of selected tree species

Table 2. Relationship (Correlation Matrix) among the biochemical parameters

	pH	Ascorbic acid	Total chlorophyll	Relative water content	Air pollution tolerance index
pH	1	0.039	-0.036	0.034	0.296**
Ascorbic Acid		1	0.147*	-0.001	0.803*
Total Chlorophyll			1	-0.171*	0.064
Relative Water Content				1	0.521**
Air Pollution Tolerance Index					1

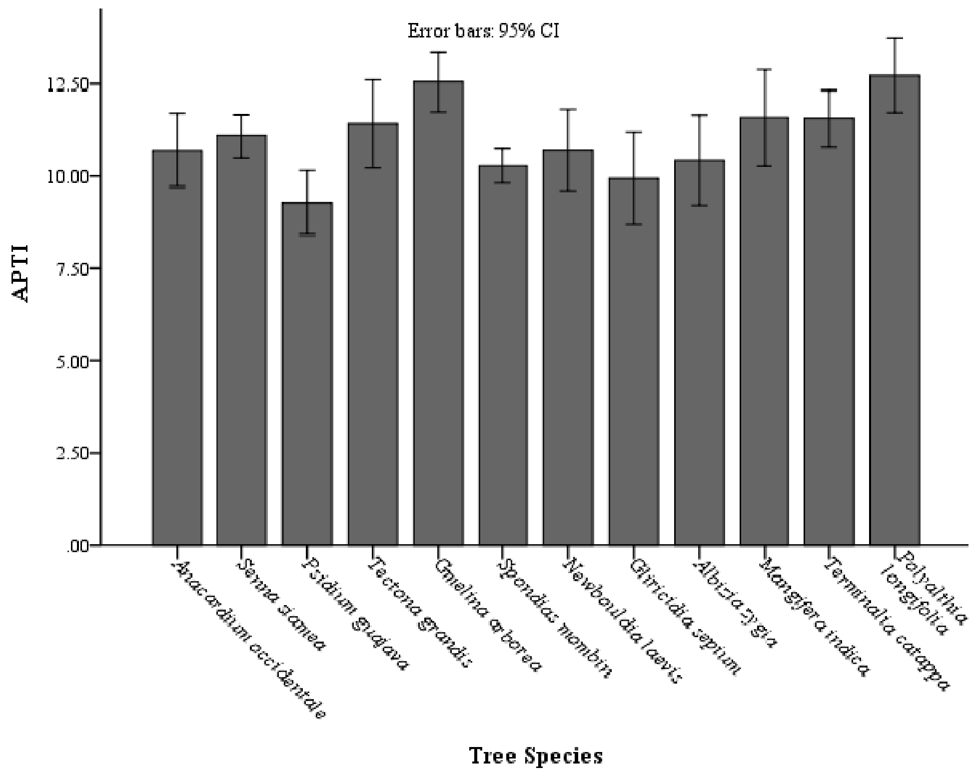

Fig. 5. Air pollution tolerance index values of the selected tree species

The result of the air pollution tolerance index (APTI) of the tree species are presented in Fig. 5 above, values were within 9.2 and 12.7. The highest value was obtained in *Polyalthia longifolia* while the lowest value was obtained in *Psidum guajava*. Tree species such as *Polyalthia longifolia*, *Ficus religiosa*, *Mangifera indica, Azadirachta indica* and *Alstonia scholaris* with high APTI values were found to be tolerant among the roadside tree species studied in a research carried out in the urban zone [28]. The present study also showed *Gmelina arborea, Polyalthia longifolia, Mangifera indica, Tectona grandis and Terminalia catappa* as species with the high APTI values. These tree species can therefore be said to have intermediate tolerance to air pollution.

4. CONCLUSION

The findings of this study revealed that *Gliricidia sepium, Spondias mombin, Senna siamea, Psiduim guajava, Anacardium occidentale, Newbouldia laevis* and *Albizia zygia* due to their low APTI values were classified as sensitive to air pollution. However, *Gmelina arborea, Polyalthia longifolia, Mangifera indica, Tectona grandis* and *Terminalia catappa* were the tolerant tree species, due to their high APTI values. Therefore, the growing of these plant species on sites exposed to continuous air pollution in Nigeria is hereby suggested.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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